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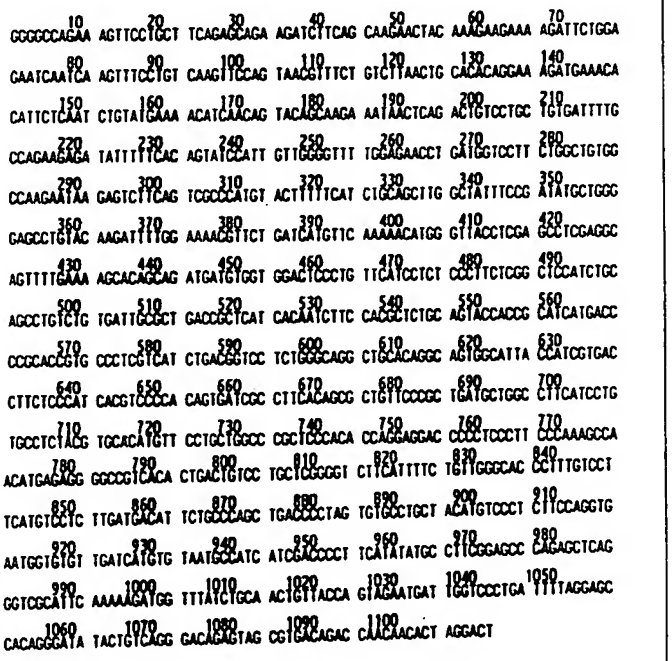
(54) Title: MAMMALIAN ADRENOCORTICOTROPIC HORMONE RECEPTORS AND USES

(57) Abstract

The present invention relates to a mammalian adrenocorticotropic hormone receptor. The invention is directed toward the isolation, characterization and pharmacological use of mammalian adrenocorticotropic hormone receptor, the gene corresponding to this receptor, a recombinant eukaryotic expression construct capable of expressing a mammalian adrenocorticotropic hormone receptor in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize mammalian adrenocorticotropic hormone receptor. The invention also provides methods for screening $ACTH^R$ agonists and antagonists *in vitro* using preparations of receptor from such cultures of eukaryotic cells transformed with a recombinant eukaryotic expression construct comprising the $ACTH^R$ receptor gene. The invention specifically provides human and bovine $ACTH^R$ genes.



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MAMMALIAN ADRENOCORTICOTROPIC HORMONE RECEPTORS AND USES

BACKGROUND OF THE INVENTION

This invention was made with government support under 1R01DK41921-03, 1R01DK43859-01, and 1P01DK44239-10A1 by the National Institutes of Health. The government has certain rights in the invention.

1. Field of the Invention

This invention relates to adrenocorticotropic hormone receptors from mammalian species and the genes corresponding to such receptors. Specifically, the invention relates to the isolation, cloning and sequencing of a human adrenocorticotropic hormone receptor gene. The invention also relates to the isolation, cloning and sequencing of a bovine adrenocorticotropic hormone receptor gene. The invention relates to the construction of eukaryotic recombinant expression constructs capable of expressing these adrenocorticotropic hormone receptors in cultures of transformed eukaryotic cells, and the production of the adrenocorticotropic hormone receptor in such cultures. The invention relates to the use of such cultures of transformed eukaryotic cells to produce homogeneous compositions of such adrenocorticotropic hormone receptors. The invention also provides culture of such cells producing adrenocorticotropic hormone receptor for the characterization of novel and useful drugs.

2. Background of the Invention

The proopiomelanocortin (POMC) gene product is processed to produce a large number of biologically active peptides. Two of these peptides, alpha-adrenocorticotropic hormone (αMSH), and adrenocorticotropic hormone (ACTH) have well understood roles in control of melanocyte and adrenocortical function, respectively. Both of these hormones, however, are found in a variety of forms with unknown functions. The melanocortin peptides also have a diverse array of biological activities in other tissues, including the brain, and immune system, and bind to specific receptors there with a distinct pharmacology [*see*,

Hanneman *et al.*, in *Peptide Hormone as Prohormones*, G. Martinez, ed. (Ellis Horwood Ltd.: Chichester, UK) pp. 53-82; DeWied & Jolles, 1982, Physiol. Rev. 62: 976-1059 for reviews].

A complete understanding of these peptides and their diverse biological activities requires the isolation and characterization of their corresponding receptors. Some biochemical studies have been reported on the prior art.

Oelofsen & Ramachandran, 1983, Arch. Biochem. Biophys. 225: 414-421 disclose receptor binding studies on ACTH receptors on rat adipocytes.

Mertz & Catt, 1991, Proc. Natl. Acad. Sci. USA 88: 8525-8529 disclose functional expression of ACTH receptors in *Xenopus laevis* oocytes following injection of total cellular RNA from adrenal tissue.

Moore *et al.*, 1991, Endocrinology 34: 107-114 relates to Allgrove syndrome, an autosomal recessive syndrome characterized by ACTH insensitivity.

The present invention comprises a human adrenocorticotropic hormone receptor gene, the nucleotide sequence of this gene and the deduced amino acid sequence of its cognate protein, a homogeneous composition of the adrenocorticotropic hormone receptor, nucleic acid hybridization probes and a method for determining the tissue distribution of expression of the gene, a recombinant expression construct capable of expressing the gene in cultures of transformed eukaryotic cells, and such cultures of transformed eukaryotic cells useful in the characterization of novel and useful drugs. The present invention also comprises the bovine adrenocorticotropic hormone receptor gene.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the nucleotide sequence of the human (SEQ ID NO:3) and bovine (SEQ ID NO:5) adrenocorticotropic hormone receptor.

Figure 2 presents an amino acid sequence comparison between the human adrenocorticotropic hormone receptor protein and the mouse and human melanocyte stimulating hormone receptor proteins.

Figure 3 illustrates the tissue distribution of human adrenocorticotropic hormone receptor gene expression by Northern blot hybridization.

Figure 4 illustrates localization of the putative ACTH receptor mRNA to the adrenal cortex by *in situ* hybridization.

SUMMARY OF THE INVENTION

The present invention relates to the cloning, expression and functional characterization of mammalian adrenocorticotropic hormone receptor ($ACTH^R$) genes. The invention comprises the nucleotide sequence of these genes encoding the mammalian $ACTH^R$s and the deduced amino acid sequences of the cognate proteins, as well as tissue distribution patterns of expression of these genes.

In particular, the present invention is directed toward the isolation, characterization and pharmacological use of the human $ACTH^R$, the gene corresponding to this receptor, a nucleic acid hybridization probe comprising DNA sequences of the human $ACTH^R$, a recombinant eukaryotic expression construct capable of expressing the human $ACTH^R$ in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize the human $ACTH^R$, a homogeneous composition of the human $ACTH^R$, and antibodies against and epitopes of the human $ACTH^R$.

The present invention is also directed toward the isolation, characterization and pharmacological use of the bovine $ACTH^R$, the gene corresponding to this receptor, a nucleic acid hybridization probe comprising DNA sequences of the bovine $ACTH^R$, a recombinant eukaryotic expression construct capable of expressing the bovine $ACTH^R$ in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize the bovine $ACTH^R$, a homogeneous composition of the bovine $ACTH^R$, and antibodies against and epitopes of the bovine $ACTH^R$.

It is an object of the invention to provide a nucleic acid comprising a nucleotide sequence encoding a mammalian $ACTH^R$. In a preferred embodiment of the invention, the nucleotide sequence encodes the human $ACTH^R$. In another preferred embodiment, the nucleotide sequence encodes the bovine $ACTH^R$.

The present invention includes a nucleic acid comprising a nucleotide sequence encoding a human $ACTH^R$ receptor derived from a DNA molecule isolated from a human genomic library (SEQ ID NO:5). In this embodiment of the invention, the nucleotide sequence includes 2028 nucleotides of the human $ACTH^R$ gene comprising 893 nucleotides of coding sequence, 696 nucleotides of

5' untranslated sequence and 439 nucleotides of 3' untranslated sequence.

The present invention also includes a nucleic acid comprising a nucleotide sequence encoding a bovine $ACTH^RR$ derived from a cDNA molecule isolated from a cDNA library constructed with bovine RNA (SEQ ID NO:3). In this embodiment of the invention, the nucleotide sequence includes 1106 nucleotides of the bovine $ACTH^R$ gene comprising 893 nucleotides of coding sequence, 133 nucleotides of 5' untranslated sequence and 82 nucleotides of 3' untranslated sequence.

The invention includes nucleic acids comprising nucleotide sequences of mammalian $ACTH^R$s, most preferably bovine and human $ACTH^R$s (SEQ ID NOs:3&5), and includes allelic variations of these nucleotide sequences and the corresponding $ACTH^R$ molecule, either naturally occurring or the product of *in vitro* chemical or genetic modification, each such variant having essentially the same nucleotide sequence as the nucleotide sequence of the corresponding $ACTH^R$ disclosed herein, wherein the resulting $ACTH^R$ molecule has substantially the same biological properties as the $ACTH^R$ molecule corresponding to the nucleotide sequence described herein. The term "substantially homologous to" as used in this invention encompasses such allelic variability as described in this paragraph.

The invention also includes a protein comprised of a predicted amino acid sequence for the bovine (SEQ ID NO:4) and human (SEQ ID NO:6) $ACTH^R$ deduced from the nucleotide sequence comprising the complete coding sequence of the bovine (SEQ ID NO:3) and human (SEQ ID NO:5) $ACTH^RR$ gene as described herein.

In another aspect, the invention comprises a homogeneous composition of a 34 kilodalton bovine $ACTH^R$ or derivative thereof, wherein the amino acid sequence of the $ACTH^R$ or derivative thereof comprises a sequence shown in Figure 2 (SEQ ID NO:4).

In another aspect, the invention comprises a homogeneous composition of a 34 kilodalton human $ACTH^R$ or derivative thereof, wherein the amino acid sequence of the $ACTH^R$ or derivative thereof comprises a sequence shown in Figure 2 (SEQ ID NO:6).

This invention provides both nucleotide and amino acid probes derived from these sequences. The invention includes probes isolated from either cDNA or genomic DNA clones, as well as probes made synthetically with the sequence information derived therefrom. The invention specifically includes but is not limited to oligonucleotide, nick-translated, random primed, or *in vitro* amplified probes made using cDNA or genomic clone embodying the invention, and oligonucleotide and other synthetic probes synthesized chemically using the nucleotide sequence information of cDNA or genomic clone embodiments of the invention.

It is a further object of this invention to provide sequences of mammalian ACTH^R, preferably the bovine or human ACTH^R, for use as nucleic acid hybridization probes to determine the pattern, amount and extent of expression of this receptor in various tissues of mammals, including humans. It is also an object of the present invention to provide nucleic acid hybridization probes derived from the sequences of the bovine or human ACTH^R to be used for the detection and diagnosis of genetic diseases. It is an object of this invention to provide nucleic acid hybridization probes derived from the DNA sequences of the bovine or human ACTH^R to be used for the detection of novel related receptor genes.

The present invention also includes synthetic peptides made using the nucleotide sequence information comprising cDNA or genomic clone embodiments of the invention. The invention includes either naturally occurring or synthetic peptides which may be used as antigens for the production of ACTH^R-specific antibodies, or used for competitors of the ACTH^R molecule for drug binding, or to be used for the production of inhibitors of the binding of agonists or antagonists or analogues thereof to ACTH^R molecule.

The present invention also provides antibodies against and epitopes of mammalian ACTH^{R}s, preferably bovine or human ACTH^R proteins. It is an object of the present invention to provide antibodies that is immunologically reactive to a mammalian ACTH^R protein. It is a particular object of the invention to provide a monoclonal antibodies to mammalian ACTH^R protein, most

preferably bovine or human ACTH^R protein.

It is also an object of the present invention to provide a hybridoma cell line that produces such an antibody. It is a particular object of the invention to provide a hybridoma cell line that is the result of fusion between a non-immunoglobulin producing bovine myeloma cell line and spleen cells derived from a bovine immunized with a human cell line which expresses ACTH^R antigen. The present invention also provides a hybridoma cell line that produces such an antibody, and that can be injected into a living bovine to provide an ascites fluid from the bovine that is comprised of such an antibody.

The present invention also provides a pharmaceutical composition comprising a therapeutically effective amount of a monoclonal antibody that is immunologically reactive to a mammalian ACTH^R, preferably a bovine or human ACTH^{R}R, and in a pharmaceutically acceptable carrier.

It is a further object of the present invention to provide an epitope of a mammalian ACTH^R protein wherein the epitope is immunologically reactive to an antibody specific for the mammalian ACTH^R. In preferred embodiments, the epitope is derived from bovine of human ACTH^R protein.

It is another object of the invention to provide a chimeric antibody that is immunologically reactive to a mammalian ACTH^R protein. In a preferred embodiment, the chimeric antibody is a monoclonal antibody. In a preferred embodiment, the ACTH^R is a bovine or human ACTH^R.

The present invention provides a recombinant expression construct comprising the nucleotide sequence of a mammalian ACTH^R, preferably the bovine or human ACTH^R and sequences sufficient to direct the synthesis of bovine or human ACTH^R in cultures of transformed eukaryotic cells. In a preferred embodiment, the recombinant expression construct is comprised of plasmid sequences derived from the plasmid pcDNAI/neo and cDNA or genomic DNA of bovine or human ACTH^R gene. This invention includes a recombinant expression construct comprising essentially the nucleotide sequences of genomic or cDNA clones of bovine or human ACTH^R in an embodiment that provides for their expression in cultures of transformed eukaryotic cells.

It is also an object of this invention to provide cultures of transformed eukaryotic cells that have been transformed with such a recombinant expression construct and that synthesize mammalian, preferably bovine or human, $ACTH^R$ protein. In a preferred embodiment, the invention provides human 293 cells that synthesize bovine $ACTH^R$. In an additional preferred embodiment, the invention provides human 293 cells that synthesize human $ACTH^R$ protein.

The present invention also includes protein preparations of mammalian, preferably bovine or human $ACTH^R$, and preparations of membranes containing mammalian $ACTH^R$, derived from cultures of transformed eukaryotic cells. In a preferred embodiment, cell membranes containing bovine $ACTH^R$ protein are isolated from 293 cell cultures transformed with a recombinant expression construct that directs the synthesis of bovine $ACTH^R$. In another preferred embodiment, cell membranes containing human $ACTH^R$ protein are isolated from 293 cell cultures transformed with a recombinant expression construct that directs the synthesis of human $ACTH^R$.

It also an object of this invention to provide mammalian, preferably bovine or human $ACTH^R$ for use in the *in vitro* screening of novel adenosine agonist and antagonist compounds. In a preferred embodiment, membrane preparations containing the bovine $ACTH^R$, derived from cultures of transformed eukaryotic cells, are used to determine the drug dissociation properties of various novel adenosine agonist and antagonist compounds *in vitro*. In another preferred embodiment, membrane preparations containing the human $ACTH^R$, derived from cultures of transformed eukaryotic cells, are used to determine the drug dissociation properties of various novel adenosine agonist and antagonist compounds *in vitro*. These properties are then used to characterize such novel compounds by comparison to the binding properties of known bovine or human $ACTH^R$ agonists and antagonists.

The present invention is also useful for the *in vivo* detection of analogues of agonists or antagonists of $ACTH^R$, known or unknown, either naturally occurring or as the embodiments of a drug.

It is an object of the present invention to provide a method for the

quantitative detection of agonists or antagonists, or analogues thereof, of $ACTH^R$, known or unknown, either naturally occurring or as the embodiments of a drug. It is an additional object of the invention to provide a method to detect such agonists, antagonists, or analogues thereof in blood, saliva, semen, cerebrospinal fluid, plasma, lymph, or any other bodily fluid.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "adrenocorticotropic hormone receptor" as used herein refers to proteins substantially homologous to, and having substantially the same biological activity as, the protein coded for by the nucleotide sequence depicted in Figure 1 (SEQ ID NO:3). This definition is intended to encompass natural allelic variations in the adrenocorticotropic hormone receptor sequence. Cloned genes of the present invention may code for $ACTH^R$s of any species of origin, including, for example, bovine, rat, rabbit, cat, and human, but preferably code for receptors of mammalian, most preferably bovine and human, origin.

Nucleic acid hybridization probes provided by the invention comprise DNA sequences that are substantially homologous to the DNA sequences in Figure 1A (SEQ ID NO:3) and 1B (SEQ ID NO:5). Nucleic acid probes are useful for detecting $ACTH^R$ gene expression in cells and tissues using techniques well-known in the art, including but not limited to Northern blot hybridization, *in situ* hybridization and Southern hybridization to reverse transcriptase - polymerase chain reaction product DNAs. The probes provided by the present invention, including oligonucleotides probes derived therefrom, are useful are also useful for Southern hybridization of mammalian, preferably human, genomic DNA for screening for restriction fragment length polymorphism (RFLP) associated with certain genetic disorders.

The production of proteins such as the $ACTH^R$ from cloned genes by genetic engineering is well known. *See, e.g.*, U.S. Patent No. 4,761,371 to Bell

et al. at Col. 6 line 3 to Col. 9 line 65. (The disclosure of all U.S. patent references cited herein is to be incorporated herein by reference.) The discussion which follows is accordingly intended as an overview of this field, and is not intended to reflect the full state of the art.

DNA which encodes the ACTH^R may be obtained, in view of the instant disclosure, by chemical synthesis, by screening reverse transcripts of mRNA from appropriate cells or cell line cultures, by screening genomic libraries from appropriate cells, or by combinations of these procedures, as illustrated below. Screening of mRNA or genomic DNA may be carried out with oligonucleotide probes generated from the ACTH^R gene sequence information provided herein. Probes may be labeled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with know procedures and used in conventional hybridization assays, as described in greater detail in the Examples below. In the alternative, ACTH^R gene sequences may be obtained by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers being produced from the ACTH^{R}R gene sequence provided herein. *See* U.S. Patent Nos. 4,683,195 to Mullis *et al.* and 4,683,202 to Mullis.

The ACTH^R may be synthesized in host cells transformed with a recombinant expression construct comprising a DNA sequence encoding the ACTH^R. Such a recombinant expression construct can also be comprised of a vector that is a replicable DNA construct. Vectors are used herein either to amplify DNA encoding the ACTH^R and/or to express DNA which encodes the ACTH^R. For the purposes of this invention, a recombinant expression construct is a replicable DNA construct in which a DNA sequence encoding the ACTH^R is operably linked to suitable control sequences capable of effecting the expression of the ACTH^R in a suitable host. The need for such control sequences will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of

transcription and translation. Amplification vectors do not require expression control domains. All that is needed is the ability to replicate in a host. usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants.

Vectors useful for practicing the present invention include plasmids, viruses (including phage), retroviruses, and integratable DNA fragments (i.e., fragments integratable into the host genome by homologous recombination). The vector replicates and functions independently of the host genome, or may, in some instances, integrate into the genome itself. Suitable vectors will contain replicon and control sequences which are derived from species compatible with the intended expression host. A preferred vector is the plasmid pcDNAI/neo. Transformed host cells are cells which have been transformed or transfected with recombinant expression constructs made using recombinant DNA techniques and comprising a mammalian $ACTH^R$. Transformed host cells may ordinarily express the mammalian $ACTH^R$, but host cells transformed for purposes of cloning or amplifying nucleic acid hybridization probe DNA need not express the receptor. When expressed, the mammalian $ACTH^R$ will typically be located in the host cell membrane.

DNA regions are operably linked when they are functionally related to each other. For example: a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of leaders sequences, contiguous and in the same translational reading frame.

Cultures of cells derived from multicellular organisms are a desirable host for recombinant $ACTH^R$ synthesis. In principal, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. However, mammalian cells are preferred, as illustrated in the Examples. Propagation of such cells in cell culture has become a routine procedure. *See* Tissue Culture, Academic Press, Kruse & Patterson, editors (1973). Examples of useful host cell lines are human 293 cells, VERO and HeLa cells, Chinese hamster ovary (CHO)

cell lines, and WI138, BHK, COS-7, CV, and MDCK cell lines. Human 293 cells are preferred. Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located upstream from the gene to be expressed, along with a ribosome binding site, RNA splice sites (if intron-containing genomic DNA is used), a polyadenylation site, and a transcriptional termination sequence.

An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral source (e.g., polyoma, adenovirus, VSV, or MPV), or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter may be sufficient.

The invention provides homogeneous compositions of mammalian $ACTH^R$ protein produced by transformed eukaryotic cells as provided herein. Such homogeneous compositions are intended to be comprised of mammalian $ACTH^R$ protein that comprises 90% of the protein in such homogenous composition.

Mammalian $ACTH^R$ protein made from cloned genes in accordance with the present invention may be used for screening agonist compounds for $ACTH^R$ activity, or for determining the amount of a $ACTH^R$ agonist or antagonist drug in a solution (e.g., blood plasma or serum). For example, host cells may be transformed with a recombinant expression construct of the present invention, $ACTH^R$ expressed in that host, the cells lysed, and the membranes from those cells used to screen compounds for $ACTH^R$ binding activity. Competitive binding assays in which such procedures may be carried out are well known in the art. By selection of host cells which do not ordinarily express $ACTH^R$s, pure preparations of membranes containing $ACTH^R$s can be obtained. Further, $ACTH^R$ agonists and antagonists can be identified by transforming host cells with vectors of the present invention. Membranes obtained from such cells can be used in binding studies wherein the drug dissociation activity is monitored.

The recombinant expression constructs of the present invention are useful in molecular biology to transform cells which do not ordinarily express the $ACTH^R$ to thereafter express this receptor. Such cells are useful as intermediates

for making cell membrane preparations useful for receptor binding assays, which are in turn useful for drug screening. Further, genes and vectors comprising the recombinant expression construct of the present invention are useful in gene therapy. For such purposes, retroviral vectors as described in U.S. Patent No. 4,650,764 to Temin & Watanabe or U.S. Patent No. 4,861,719 to Miller may be employed. Cloned genes of the present invention, or fragments thereof, may also be used in gene therapy carried out homologous recombination or site-directed mutagenesis. *See generally* Thomas & Capecchi, 1987, Cell 51: 503-512; Bertling, 1987, Bioscience Reports 7: 107-112; Smithies *et al.*, 1985, Nature 317: 230-234.

Oligonucleotides of the present invention are useful as diagnostic tools for probing ACTH-receptor gene expression in tissues. For example, tissues can be probed *in situ* with oligonucleotide probes carrying detectable groups by conventional autoradiography techniques, as explained in greater detail in the Examples below, to investigate native expression of this receptor or pathological conditions relating thereto. Further, chromosomes can be probed to investigate the presence or absence of the $ACTH^R$ gene, and potential pathological conditions related thereto, as also illustrated by the Examples below.

The invention also provides antibodies that are immunologically reactive to a mammalian $ACTH^R$. The antibodies provided by the invention can be raised in animals by inoculation with cells that express a mammalian $ACTH^RR$ or epitopes of a mammalian $ACTH^R$ using methods well known in the art. Animals that can be used for such inoculations include individuals from species comprising cows, sheep, pigs, mice, rats, rabbits, hamsters, goats and primates. Preferred animals for inoculation are rodents (including mice, rats, hamsters) and rabbits. The most preferred animal is the mouse.

Cells that can be used for such inoculations, or for any of the other means used in the invention, include any cell line which naturally expresses a mammalian $ACTH^R$, or any cell or cell line that expresses a mammalian $ACTH^R$ or any epitope therein as a result of molecular or genetic engineering, or that has been treated to increase the expression of a mammalian $ACTH^R$ by physical,

biochemical or genetic means. Preferred cells are human cells. most preferably human 293 cells that have been transformed with a recombinant expression construct comprising DNA sequences encoding a mammalian $ACTH^R$ and that express the mammalian $ACTH^R$ gene product.

The present invention provides monoclonal antibodies that are immunologically reactive with an epitope that is a mammalian $ACTH^R$ present on the surface of mammalian cells, preferably human or bovine cells. These antibodies are made using methods and techniques well known to those of skill in the art.

Monoclonal antibodies provided by the present invention are produced by hybridoma cell lines, that are also provided by the invention and that are made by methods well known in the art. Hybridoma cell lines are made by fusing individual cells of a myeloma cell line with spleen cells derived from animals immunized with cells expressing a mammalian $ACTH^R$, including human cells, as described above. The myeloma cell lines used in the invention include lines derived from myelomas of mice, rats, hamsters, primates and humans. Preferred myeloma cell lines are from bovine, and the most preferred bovine myeloma cell line is P3X63-Ag8.653. The animals from whom spleens are obtained after immunization are rats, mice and hamsters, preferably mice, most preferably Balb/c mice. Spleen cells and myeloma cells are fused using a number of methods well known in the art, including but not limited to incubation with inactivated Sendai virus and incubation in the presence of polyethylene glycol (PEG). The most preferred method for cell fusion is incubation in the presence of a solution of 45% (w/v) PEG-1450. Monoclonal antibodies produced by hybridoma cell lines can be harvested from cell culture supernatant fluids from *in vitro* cell growth; alternatively, hybridoma cells can be injected subcutaneously and/or into the peritoneal cavity of an animal, most preferably a bovine, and the monoclonal antibodies obtained from blood and/or ascites fluid.

Monoclonal antibodies provided by the present invention can also be produced by recombinant genetic methods well known to those of skill in the art, and the present invention encompasses antibodies made by such methods that are

immunologically reactive with an epitope of a mammalian ACTH^R.

The present invention encompasses fragments of the antibody that are immunologically reactive with an epitope of a mammalian ACTH^R. Such fragments can be produced by any number of methods, including but not limited to proteolytic cleavage, chemical synthesis or preparation of such fragments by means of genetic engineering technology. The present invention also encompasses single-chain antibodies that are immunologically reactive with an epitope of a mammalian ACTH^R made by methods known to those of skill in the art.

The present invention also encompasses an epitope of a mammalian ACTH^R that is comprised of sequences and/or a conformation of sequences present in the mammalian ACTH^R molecule. This epitope may be naturally occurring, or may be the result of proteolytic cleavage of the mammalian ACTH^R molecule and isolation of an epitope-containing peptide or may be obtained by synthesis of an epitope-containing peptide using methods well known to those skilled in the art. The present invention also encompasses epitope peptides produced as a result of genetic engineering technology and synthesized by genetically engineered prokaryotic or eukaryotic cells.

The invention also includes chimeric antibodies, comprised of immunologically reactive light chain and heavy chain peptides to an epitope that is a mammalian ACTH^R. The chimeric antibodies embodied in the present invention include those that are derived from naturally occurring antibodies as well as chimeric antibodies made by means of genetic engineering technology well known to those of skill in the art.

The Examples which follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

EXAMPLE 1

Isolation of an ACTH Receptor Probe by Random PCR Amplification of Human Melanoma cDNA Using Degenerate Oligonucleotide Primers

In order to clone novel G-protein coupled receptors, human melanoma cDNA was used as template for a polymerase chain reaction (PCR)-based random cloning experiment. PCR was performed using a pair of degenerate oligonucleotide primers corresponding to the putative third and sixth transmembrane regions of G-protein coupled receptors (Libert *et al.*, 1989, Science 244: 569-72; Zhou *et al.*, 1990, Nature 347: 76-80). The PCR products obtained in this experiment were characterized by nucleotide sequencing. Two novel sequences representing novel G-protein-coupled receptors were identified.

PCR amplification was performed as follows. Total RNA was isolated from a human melanoma tumor sample by the guanidinium thiocyanate method (Chirgwin *et al.*, 1979, Biochemistry 18: 5294-5299). Double-stranded cDNA was synthesized from total RNA with murine reverse transcriptase (BRL, Gaithersburg, MD) by oligo-dT priming [Maniatis *et al.*, Molecular Cloning: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), 1990]. The melanoma cDNA mixture was then subjected to 45 cycles of PCR amplification using 500 picomoles of degenerate oligonucleotide primers having the following sequence:

Primer III (sense):

GAGTCGACCTGTG(C/T)G(C/T)(C/G)AT(C/T)(A/G)CIIT(G/T)GAC(C/A)G(C/G)TAC

(SEQ ID NO:1)

and

Primer VI (antisense):

CAGAATTCAG(T/A)AGGGCAICCAGCAGAI(G/C)(G/A)(T/C)GAA
(SEQ ID NO:2)

in 100 μl of a solution containing 50 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$,

0.01% gelatin, 200 μM each dNTP, and 2.5 units of *Taq* polymerase (Saiki *et al.*, 1988, Science 239: 487-491). These primers were commercially synthesized by Research Genetics Inc. (Huntsville, AL). Each PCR amplification cycle consisted of incubations at 94°C for 1 min (denaturation), 45°C for 2 min (annealing), and 72°C for 2 min (extension).

Amplified products of the PCR reaction were extracted with phenol/chloroform and precipitated with ethanol. After digestion with *Eco*RI and *Sal*I, the PCR products were separated on a 1.2% agarose gel. A slice of this gel, corresponding to PCR products of 300 basepairs (bp) in size, was cut out and purified using glass beads and sodium iodide, and then the insert was cloned into a pBKS cloning vector (Stratagene, LaJolla, CA).

A total of 172 of such pBKS clones containing inserts were sequenced using Sequenase (U. S. Biochemical Corp., Cleveland, OH) by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977, Proc. Natl. Acad. Sci. USA 74: 5463-5467). Two types of sequences homologous to other G-protein coupled receptors were identified.

EXAMPLE 2

Isolation and Characterization of Human ACTH^R Genomic Clones

In order to isolate the human gene corresponding to one of the two G-protein coupled receptor probes of Example 1, a human genomic library was screened at high stringency (50% formamide, 1M NaCl, 50nM Tris-HCl, pH 7.5, 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate, 100μg/ml salmon sperm DNA, 10X Denhardt's solution, 42°C), using the human PCR fragments isolated as described in Example 1. Two different types of sequences were isolated, corresponding to the two PCR fragments, and were found to encode highly related G protein coupled receptors. These genomic clones were sequenced as described in Example 1. The nucleotide sequence of this clone is shown in Figure 1A (SEQ ID NO:3). Nucleotide sequence analysis and homology comparisons were done on the OHSU computer system with software provided by Intelligenetics Inc. (Mountain View, CA).

One of these genomic clones was determined to encode an human MSH receptor (*see* co-pending U.S. Patent Application Serial No. 07/866,979, filed April 10, 1992, hereby incorporated by reference). The human MSH receptor has a predicted amino acid sequence that is 75% identical and colinear with a mouse αMSH receptor cDNA sequence.

The second human genomic clone isolated encodes a highly related G-coupled receptor protein (SEQ ID NO:3). The predicted amino acid sequence (SEQ ID NO:4) of this clone (Figure 2, represented as human ACTH-R) is 39% identical and also colinear, excluding the third intracellular loop and carboxy-terminal tail, with the human MSH receptor gene product (Figure 2; represented as human MSH-R). The predicted molecular weight of this putative $ACTH^R$ is 33.9 kilodaltons (kD). Based on its high degree of homology to the murine (mouse MSH-R; Figure 2) and human MSH receptors, and the pattern of expression in different tissue types, as described in Example 3 below, this gene is a believed to encode a human ACTH receptor.

A bovine genomic DNA clone was isolated from a bovine genomic library, essentially as described above, and its nucleotide sequence determined (Figure 1B; SEQ ID NO:5).

The predicted amino acid sequences of the mouse αMSH^R, human MSH^R, and the putative human $ACTH^R$ are aligned in Figure 2. These sequences define the melanocortin receptors as a novel subfamily of the G protein-coupled receptors with a number of unusual features. The melanocortin receptors are the smallest G protein-coupled receptors identified to date (297-317aa) resulting from a short amino terminal extracellular domain, a short carboxy-terminal intracellular domain, and a very small third intracellular loop. The melanocortin receptors are lack several amino acid residues present in most G protein coupled receptors (*see* Probst *et al.*, 1992, DNA & Cell Biol. 11: 1-20), including the proline residues in the 4th and 5th transmembrane domains, likely to introduce a bend in the alpha helical structure of the transmembrane domains and thought to be involved in the formation of the binding pocket (*see* Applebury & Hargrave, 1986, Vision Res. 26: 1881-1895), and one or both of the cysteine residues thought to form a

disulfide bond between the first and second extracellular loops (*see* Dixon *et al.*, 1987, EMBO J. 6: 3269-3275 and Karnik *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85: 8459-8463). Remarkably, the melanocortin receptors do not appear highly related to the other G protein-coupled receptors which recognize peptide ligands, such as the receptors for bombesin (*see* Spindel *et al.*, 1990, Mol. Endocrinol. 4: 1956-1963) or substance K (*see* Masu *et al.*, 1987, Nature 329: 836-838), but rather, are more closely related to the receptor for Δ^9-tetradhydrocannabinol (*see* Matsuda *et al.*, 1990, Nature 346: 561-564). For example, the human ACTH^R and rat cannabinoid receptors are about 30% identical in predicted transmembrane and intracellular loop amino acid sequences. The cannabinoid receptor also lacks the conserved proline in transmembrane 5 and the cysteine in the first extracellular loop necessary for disulfide bond formation. Least parsimony analysis with the receptor sequences shown in Figure 2 suggests the cannabinoid and melanocortin receptors may be evolutionarily related and form a subfamily distinct from the peptide receptors and the amine receptors. Regardless of whether the similarities are the result of evolutionary conservation or convergence, the sequence and putative structural similarities between the melanocortin and cannabinoid receptors may be informative in the search for the endogenous cannabinoid-like ligand.

EXAMPLE 3

Tissue Distribution of ACTH Receptor Gene Expression

To further gain insight into this receptor, we have examined the tissue distribution of its corresponding mRNA from various tissues by performing Northern hybridization experiments on RNA isolated from various tissues (*see* Maniatis *et al.*, *ibid.*). The results of these experiments are shown in Figure 3.

A panel of tissue samples was examined by Northern hybridization analysis performed under high stringency conditions. The nitrocellulose filter was hybridized with a putative human ACTH receptor probe to determine the distribution of receptor mRNA. In two primary human melanocyte cultures

examined, the $ACTH^R$ is encoded by two mRNA species of approximately equal stoichiometry, one at 3.0kb, and one which co-migrates with murine αMSH^R mRNA at 3.9kb.

The putative human ACTH receptor is encoded predominantly by a single mRNA species of approximately 4.0kb in the human adrenal gland, although several minor species are present as well. Northern analysis of a panel of tissues from the rhesus macaque performed under high stringency conditions demonstrated the existence of a cross-reacting 4.0kb species specific to the rhesus adrenal gland (Fig 3). *In situ* hybridization of a fragment of the putative human ACTH receptor to sections of rhesus adrenal tissue localized the expression of this receptor solely to the cortex, with no apparent hybridization to the medulla or capsule, as would be predicted for this receptor (Figure 4). Adrenal tissue from a juvenile rhesus macaque was fixed for 24 hours in 10% formalin in phosphate buffered saline, then incubated for 24 hours in 20% sucrose in PBS. Sections were prepared and hybridized with a 600 nucleotide ^{35}S-labelled RNA antisense probe complementary to coding sequence spanning transmembrane domains 1-6 of the putative human ACTH receptor. Hybridizations were performed at 65°C in 2xSSC and washed at 65°C with 0.1xSSC.

The results of these experiments are shown in Figure 4. Panel A illustrates lightfield micrograph of an hematoxylin and eosin stained section of rhesus adrenal showing capsule (C), zona glomerulosa (G), zona fasciculata (F), zona reticulata (R), and medulla (M). Panel B depicts darkfield micrograph of the same field. Within the cortex, receptor expression was found across the entire *zona fasciculata*, the site of glucocorticoid production, and in the cortical half of the *zona glomerulosa*, the site of aldosterone synthesis. The *zona reticulata* was largely negative, except for a small band of hybridization adjacent to the medulla, which might result from a cross-reaction between the putative $ACTH^R$ probe and a receptor for γ_3MSH, which is known to bind to this region of the adrenal cortex.

Additionally, we have been unable to detect expression in the brain of ACTH receptor described here, despite extensive documentation of ACTH binding

sites there as well as in other tissues. These finding suggest the existence of alternate forms of these or related receptors that may be specifically expressed in brain tissue.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

WHAT WE CLAIM IS:

1. A nucleic acid comprising a nucleotide sequence encoding a mammalian adrenocorticotropic hormone receptor.

2. A nucleic acid according to Claim 1 wherein the mammalian adrenocorticotropic hormone receptor is the bovine adrenocorticotropic hormone receptor.

3. A nucleic acid according to Claim 1 wherein the nucleotide sequence is substantially homologous to the sequence in Figure 1A (SEQ ID NO:3).

4. A nucleic acid according to Claim 1 wherein the mammalian adrenocorticotropic hormone receptor is the human adrenocorticotropic hormone receptor.

5. A nucleic acid according to Claim 1 wherein the nucleotide sequence is substantially homologous to the sequence in Figure 1B (SEQ ID NO:5).

6. A nucleic acid according to Claim 1 wherein the mammalian adrenocorticotropic hormone receptor encoded therein has the biological properties of a native adrenocorticotropic hormone receptor.

7. A homogeneous composition of a 33.9 kilodalton adrenocorticotropic hormone receptor or derivative thereof, wherein the amino acid sequence of the adrenocorticotropic hormone receptor or derivative thereof comprises the sequence shown in Figure 2 (SEQ ID NO:4).

8. A homogeneous composition of a 33.9 kilodalton adrenocorticotropic hormone receptor or derivative thereof, wherein the amino acid sequence of the adrenocorticotropic hormone receptor or derivative thereof comprises the sequence shown as SEQ ID NO:6.

9. A nucleic acid hybridization probe for the detection of mammalian adrenocorticotropic hormone receptor expression comprising the nucleotide sequence of Claim 3.

10. The nucleic acid hybridization probe according to Claim 9 whereby the probe is adapted for use in the detection and diagnosis of genetic disease in

a human.

11. The nucleic acid hybridization probe according to Claim 9 whereby the probe is adapted for use in the detection, isolation and characterization of novel mammalian receptor genes.

12. A nucleic acid hybridization probe for the detection of mammalian adrenocorticotropic hormone receptor expression comprising the nucleotide sequence of Claim 5.

13. The nucleic acid hybridization probe according to Claim 12 whereby the probe is adapted for use in the detection and diagnosis of genetic disease in a human.

14. The nucleic acid hybridization probe according to Claim 12 whereby the probe is adapted for use in the detection, isolation and characterization of novel mammalian receptor genes.

15. A recombinant expression construct comprising a nucleotide sequence encoding a mammalian adrenocorticotropic hormone receptor.

16. A recombinant expression construct comprising the DNA sequence of Claim 3, wherein the construct is capable of expressing the bovine adrenocorticotropic hormone receptor in a transformed eukaryotic cell culture.

17. A recombinant expression construct comprising the DNA sequence of Claim 5, wherein the construct is capable of expressing the human adrenocorticotropic hormone receptor in a transformed eukaryotic cell culture.

18. The recombinant expression construct of Claim 15 comprising pcDNAI/neo sequences.

19. A eukaryotic cell culture transformed with the expression construct of Claim 16, wherein the transformed eukaryotic cell culture is capable of expressing bovine adrenocorticotropic hormone receptor.

20. A eukaryotic cell culture transformed with the expression construct of Claim 17, wherein the transformed eukaryotic cell culture is capable of expressing the human adrenocorticotropic hormone receptor.

21. A method of screening a compound as an inhibitor of agonist binding to a mammalian adrenocorticotropic hormone receptor, the method

comprising the following steps:

(a) transforming a eukaryotic cell culture with an expression construct as in Claim 15 capable of expressing the adrenocorticotropic hormone receptor in a eukaryotic cell; and

(b) assaying for ability of the compound to inhibit the binding of a detectable adrenocorticotropic hormone receptor agonist.

22. The method of Claim 21 wherein the mammalian adrenocorticotropic hormone receptor is the bovine adrenocorticotropic hormone receptor.

23. The method of Claim 21 wherein the mammalian adrenocorticotropic hormone receptor is the human adrenocorticotropic hormone receptor.

24. A method of quantitatively detecting a compound as an inhibitor of agonist binding to a mammalian adrenocorticotropic hormone receptor, the method comprising the following steps:

(a) transforming a eukaryotic cell culture with an expression construct as in Claim 15 capable of expressing the mammalian adrenocorticotropic hormone receptor in a eukaryotic cell; and

(b) assaying for amount of a compound by measuring the extent of inhibition of binding of a detectable receptor agonist.

25. The method of Claim 24 wherein the mammalian adrenocorticotropic hormone receptor is the bovine adrenocorticotropic hormone receptor.

26. The method of Claim 24 wherein the mammalian adrenocorticotropic hormone receptor is the human adrenocorticotropic hormone receptor.

27. The method of Claim 24 wherein the compound to be tested is present in a human.

28. The method of Claim 24 wherein the compound is present in human blood.

29. The method of Claim 24 wherein the compound is present in human

cerebrospinal fluid.

30. The method of Claim 24 wherein the compound is unknown.

31. An antibody or fragment thereof that is immunologically reactive to a mammalian adrenocorticotropic hormone receptor.

32. The antibody according to Claim 31, wherein the antibody is a monoclonal antibody.

33. The antibody according to Claim 31, wherein the mammalian adrenocorticotropic hormone receptor is the bovine adrenocorticotropic hormone receptor.

34. The antibody according to Claim 31, wherein the mammalian adrenocorticotropic hormone receptor is the human adrenocorticotropic hormone receptor.

35. A cell line which produces an antibody or fragment thereof that is immunologically reactive to a mammalian adrenocorticotropic hormone receptor.

36. The cell line according to Claim 35, wherein the antibody is a monoclonal antibody.

37. The cell line according to Claim 35, wherein the mammalian adrenocorticotropic hormone receptor is the bovine adrenocorticotropic hormone receptor.

38. The cell line according to Claim 35, wherein the mammalian adrenocorticotropic hormone receptor is the human adrenocorticotropic hormone receptor.

39. A pharmaceutical composition comprising a therapeutically effective amount of an antibody or fragment thereof according to claim 31 in a pharmaceutically acceptable carrier.

40. An epitope of a mammalian adrenocorticotropic hormone receptor wherein the epitope is immunologically reactive to the antibody or fragment thereof according to claim 31.

41. The epitope according to claim 40 wherein the mammalian adrenocorticotropic hormone receptor is the bovine adrenocorticotropic hormone receptor.

42. The epitope according to claim 40 wherein the mammalian adrenocorticotropic hormone receptor is the human adrenocorticotropic hormone receptor.

43. The antibody of Claim 31 that is a chimeric antibody.

44. The chimeric antibody according to claim 43 wherein the mammalian adrenocorticotropic hormone receptor is the bovine adrenocorticotropic hormone receptor.

45. The chimeric antibody according to claim 43 wherein the mammalian adrenocorticotropic hormone receptor is the human adrenocorticotropic hormone receptor.

FIG. 1A

```
        10         20         30         40         50         60         70
ACAACACTTT ATATATATTT TTATAAATGT AAGGGGTACA AARGTGCCAT TTTGTTACAT GGATATACCG

        80         90        100        110        120        130        140
TGTAGTGGTG AAGCCTGGGC TTTTAGTGTA TCTGTCATCA GAATAACATA CGTGTTACCC ATAGGAATTT

       150        160        170        180        190        200        210
CTCATCACCC GCCCCCTCCA CCCTTCGAGT CTCCAATGTC CATTCCACAC TCTATATCCA CGTGTATGCA

       220        230        240        250        260        270        280
TATAGCTCCA CATATAAGTG AGAACATGTA GTATTTGACT TCCTCTTTCT GAGTTATTTC ACTTTGATAA

       290        300        310        320        330        340        350
TGGCCTCCAC TTCCATCCAT GTTGCTGCAA AAGACATGAC CTTATTCTTT TTGATAGCTG GGGAGTACTC

       360        370        380        390        400        410        420
CATTGTGTAT ATGTACCACA TTTNCTTTAT CCATTCACCC ATTGANGAAC ACTTAGTTGA TTCCATATCT

       430        440        450        460        470        480        490
TTGCTATTGT CACTAGTGCT GCAATAAACA TACATGTGCA GGCTCCTTCT AATATACTGA TTTATATTTT

       500        510        520        530        540        550        560
ATGGAGAGAG ATAGAGTTCT TAGCGAGTGT GCTGTTTATT TCTAGTGTAC TTGCAACTAA TATTCTGTAT

       570        580        590        600        610        620        630
ACTCCCTTTA GGTGATTGGA GATTTAACTT AGATCTCCAG CAAGTGCTAC AAGAAGAAAA GATCCTGAAG

       640        650        660        670        680        690        700
AATCAATCAA GTTTCCGTGA AGTCAAGTCC AAGTAACATC CCCGCCTTAA CCACAAGCAG GAGAAATGAA

       710        720        730        740        750        760        770
GCACATTATC AACTCGTATG AAAACATCAA CAACACAGCA AGAAATAATT CCGACTGTCC TCGTGTGGTT
```

FIG. 1B

```
                780        790        800        810        820        830        840
TTGCCGGAGG AGATATTTTT CACAATTTCC ATTGTTGGAG TTTTGGAGAA TCTGATCGTC CTGCTGGCTG
                850        860        870        880        890        900        910
TGTTCAAGAA TAAGAATCTC CAGGCACCCA TGTACTTTTT CATCTGTAGC TTGGCCATAT CTGATATGCT
                920        930        940        950        960        970        980
GGGCAGCCTA TATAAGATCT TGGAAAATAT CCTGATCATA TTGAGAAACA TGGGCTATCT CAAGCCACGT
                990       1000       1010       1020       1030       1040       1050
GGCAGTTTTG AAACCACAGC CGATGACATC ATCGACTCCC TGTTTGTCCT CTCCCTGCTT GGCTCCATCT
               1060       1070       1080       1090       1100       1110       1120
TCAGCCTGTC TGTGATTGCT GCGGACCGCT ACATCACCAT CTTCCACGCA CTGCGGTACC ACAGCATCGT
               1130       1140       1150       1160       1170       1180       1190
GACCATGCGC CGCACTGTGG TGGTGCTTAC GGTCATCTGG ACGTTCTGCA CGGGGACTGG CATCACCATG
               1200       1210       1220       1230       1240       1250       1260
GTGATCTTCT CCCATCATGT GCCCACAGTG ATCACCTTCA CGTCGCTGTT CCCGCTGATG CTGGTCTTCA
               1270       1280       1290       1300       1310       1320       1330
TCCTGTGCCT CTATGTGCAC ATGTTCCTGC TGGCTCGATC CCACACCAGG AAGATCTCCA CCCTCCCCAG
               1340       1350       1360       1370       1380       1390       1400
AGCCAACATG AAAGGGGCCA TCACACTGAC CATCCTGCTC GGGGTCTTCA TCTTCTGCTG GGCCCCCTTT
               1410       1420       1430       1440       1450       1460       1470
GTGCTTCATG TCCTCTTGAT GACATTCTGC CCAAGTAACC CCTACTGCGC CTGCTACATG TCTCTCTTCC
```

FIG. 1C

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      1480       1490       1500       1510       1520       1530       1540
AGGTGAACGG CATGTTGATC ATGTGCAATG CCGTCATTGA CCCCTTCATA TATGCCTTCC GGAGCCCAGA

      1550       1560       1570       1580       1590       1600       1610
GCTCAGGGAC GCATTCAAAA AGATGATCTT CTGCAGCAGG TACTGGTAGA ATGGCTGATC CCTGGTTTTA

      1620       1630       1640       1650       1660       1670       1680
GAATCCATGG GAATAACGTT GCCAAGTGCC AGAATAGTGT AACATTCCAA CAAATGCCAG TGCTCCTCAC

      1690       1700       1710       1720       1730       1740       1750
TGGCCTTCCT TCCCTAATGG ATGCAAGGAT GACCCACCAG CTAGTGTTTC TGAATACTAT GGCCAGGAAC

      1760       1770       1780       1790       1800       1810       1820
AGTCTATTGT AGGGGCAACT CTATTTGTGA CTGGACAGAT AAAACGTGTA GTAAAAGAAG GATAGAATAC

      1830       1840       1850       1860       1870       1880       1890
AAAGTATTAG GTACAAAAGT AATTANGGTT TNNGCNATTA CTTNNMATGA CNNNAAATNG CANTTACTTT

      1900       1910       1920       1930       1940       1950       1960
TGCACCAATC TAGTAAAACA GCAATAAAAA TTCAAGGGCT TTGGGCTAAG GCAAAGACTT GCTTTCCTGT

      1970       1980       1990       2000       2010       2020
GGACATSTAA CAAGCCAGTT CTGANGGCGG CCTTTCCAGG TGGAGGCCAT TGCAGCCAAT TTCAGAGT
```

FIG. 2

```
                10                  20                  30                  40                  50                  60                  70
GGGGCCAGAA AGTTCCTGCT TCAGAGCAGA AGATCTTCAG CAAGAACTAC AAAGAAGAAA AGATTCTGGA

                80                  90                 100                 110                 120                 130                 140
GAATCAATCA AGTTTCCTGT CAAGTTCCAG TAACGTTTCT GTCTTAACTG CACACAGGAA AGATGAAACA

               150                 160                 170                 180                 190                 200                 210
CATTCTCAAT CTGTATGAAA ACATCAACAG TACAGCAAGA AATAACTCAG ACTGTCCTGC TGTGATTTTG

               220                 230                 240                 250                 260                 270                 280
CCAGAAGAGA TATTTTTCAC AGTATCCATT GTTGGGGTTT TGGAGAACCT GATGGTCCTT CTGGCTGTGG

               290                 300                 310                 320                 330                 340                 350
CCAAGAATAA GAGTCTTCAG TCGCCCATGT ACTTTTTCAT CTGCAGCTTG GCTATTTCCG ATATGCTGGG

               360                 370                 380                 390                 400                 410                 420
GAGCCTGTAC AAGATTTTGG AAAACGTTCT GATCATGTTC AAAAACATGG GTTACCTCGA GCCTCGAGGC

               430                 440                 450                 460                 470                 480                 490
AGTTTTGAAA AGCACAGCAG ATGATGTGGT GGACTCCCTG TTCATCCTCT CCCTTCTCGG CTCCATCTGC

               500                 510                 520                 530                 540                 550                 560
AGCCTGTCTG TGATTGCGCT GACCGCTCAT CACAATCTTC CACGCTCTGC AGTACCACCG CATCATGACC

               570                 580                 590                 600                 610                 620                 630
CCGCACCGTG CCCTCGTCAT CTGACGGTCC TCTGGGCAGG CTGCACAGGC AGTGGCATTA CCATCGTGAC

               640                 650                 660                 670                 680                 690                 700
CTTCTCCCAT CACGTCCCCA CAGTGATCGC CTTCACAGCG CTGTTCCCGC TGATGCTGGC CTTCATCCTG

               710                 720                 730                 740                 750                 760                 770
TGCCTCTACG TGCACATGTT CCTGCTGGCC CGCTCCCACA CCAGGAGGAC CCCCTCCCTT CCCAAAGCCA
```

FIG. 2 cont'd

```
       780        790        800        810        820        830        840
ACATGAGAGG GGCCGTCACA CTGACTGTCC TGCTCGGGGT CTTCATTTTC TGTTGGGCAC CCTTTGTCCT
       850        860        870        880        890        900        910
TCATGTCCTC TTGATGACAT TCTGCCCAGC TGACCCCTAG TGTGCCTGCT ACATGTCCCT CTTCCAGGTG
       920        930        940        950        960        970        980
AATGGTGTGT TGATCATGTG TAATGCCATC ATCGACCCCT TCATATATGC CTTCGGAGCC CAGAGCTCAG
       990       1000       1010       1020       1030       1040       1050
GGTCGCATTC AAAAAGATGG TTTATCTGCA ACTGTTACCA GTAGAATGAT TGGTCCCTGA TTTTAGGAGC
      1060       1070       1080       1090       1100
CACAGGGATA TACTGTCAGG GACAGAGTAG CGTGACAGAC CAACAACACT AGGACT
```

FIG. 3

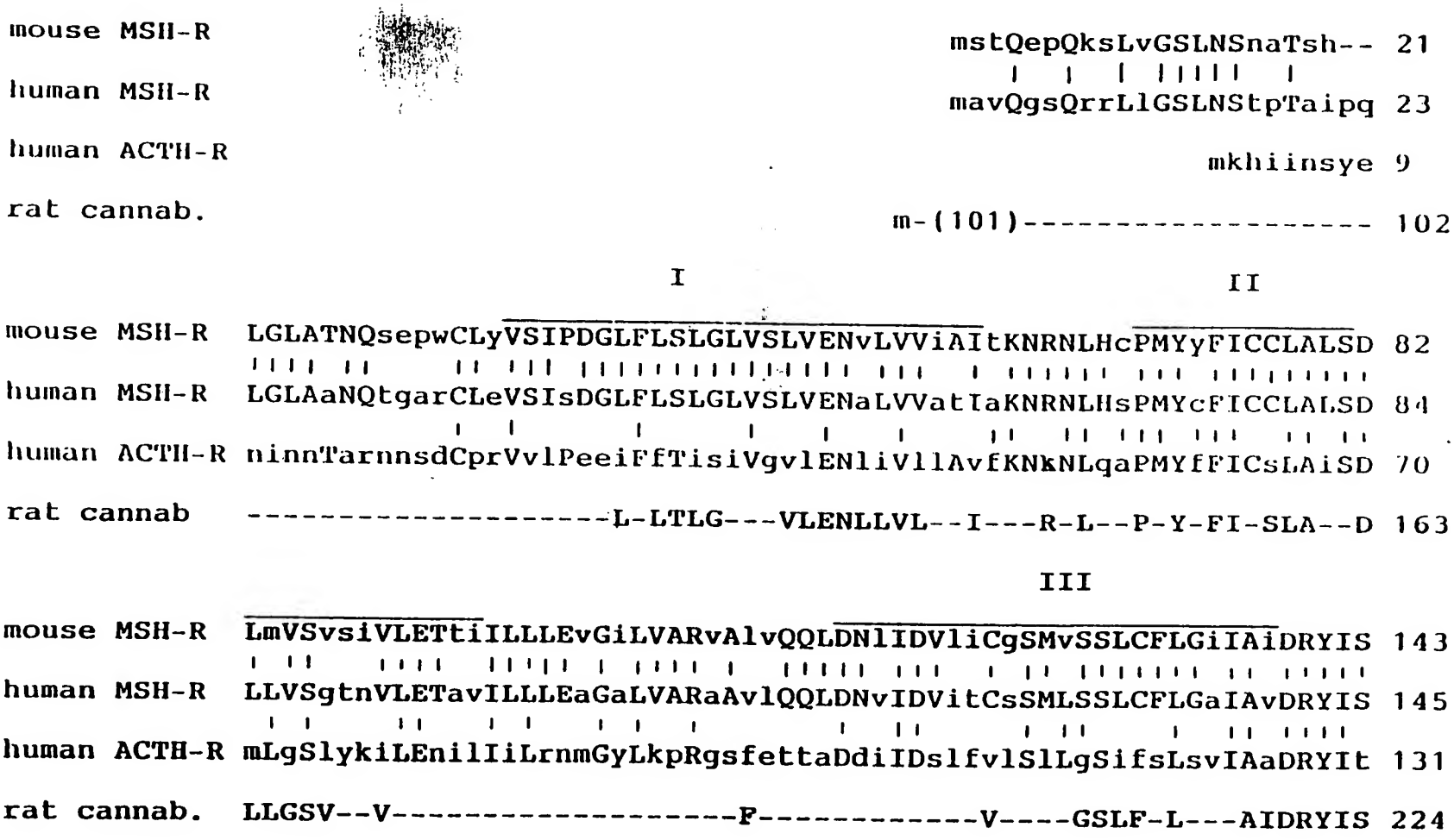

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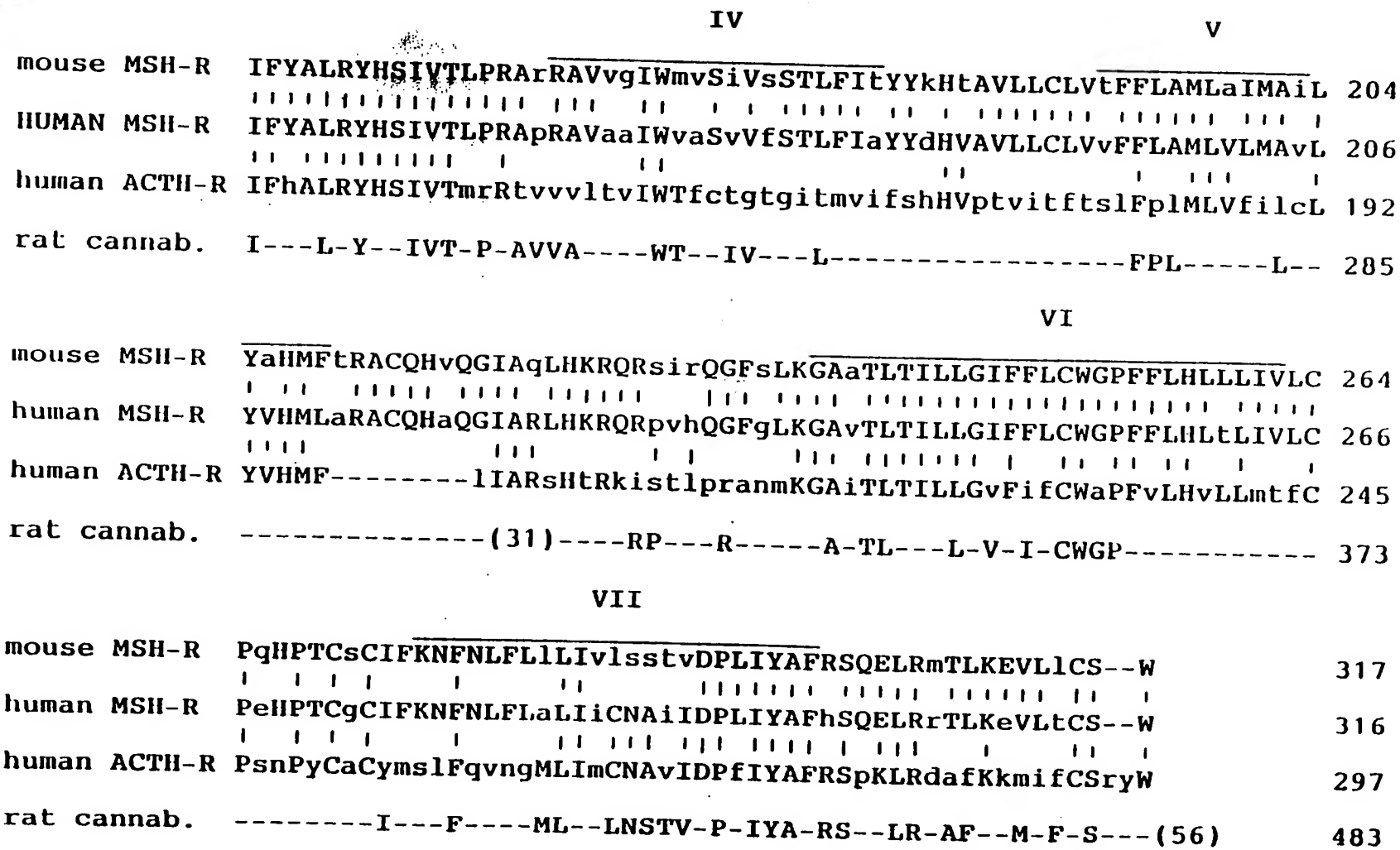
FIG. 3 cont'd


IV
V
mouse MSH-R IFYALRYHSIVTLPRArRAVvgIWmvSiVsSTLFItYYkHtAVLLCLVtFFLAMLaIMAiL 204
HUMAN MSH-R IFYALRYHSIVTLPRApRAVaaIWvaSvVfSTLFIaYYdHVAVLLCLVvFFLAMLVLMAvL 206
human ACTH-R IFhALRYHSIVTmrRtvvvltvIWTfctgtgitmvifshHVptvitftslFplMLVfilcL 192
rat cannab. I---L-Y--IVT-P-AVVA----WT--IV---L----------FPL-----L-- 285
VI
mouse MSH-R YaHMFtRACQHvQGIAqLHKRQRsirQGFsLKGAaTLTILLGIFFLCWGPFFLHLLLIVLC 264
human MSH-R YVHMLaRACQHaQGIARLHKRQRpvhQGFgLKGAvTLTILLGIFFLCWGPFFLHLtLIVLC 266
human ACTH-R YVHMF--------lIARsHtRkistlpranmKGAiTLTILLGvFifCWaPFvLHvLLmtfC 245
rat cannab. ----------(31)----RP---R-----A-TL---L-V-I-CWGP---------- 373
VII
mouse MSH-R PqHPTCsCIFKNFNLFLlLIvlsstvDPLIYAFRSQELRmTLKEVLlCS--W 317
human MSH-R PeHPTCgCIFKNFNLFLaLIiCNAiIDPLIYAFhSQELRrTLKeVLtCS--W 316
human ACTH-R PsnPyCaCymslFqvngMLImCNAvIDPfIYAFRSpKLRdafKkmifCSryW 297
rat cannab. --------I---F----ML--LNSTV-P-IYA-RS--LR-AF--M-F-S---(56) 483

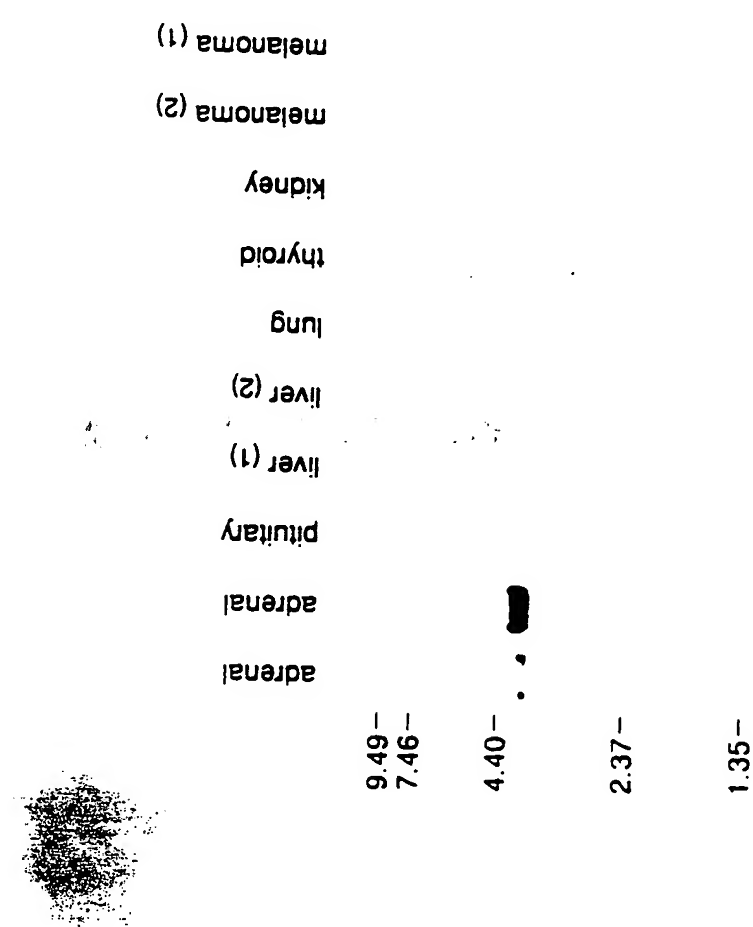


Figure 4

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Figure 5

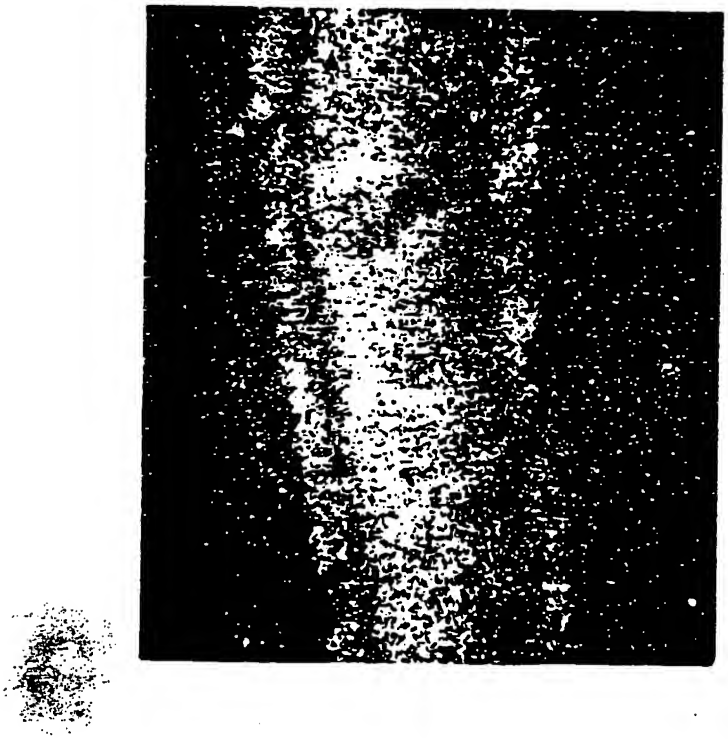

Figure 6

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/03246

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C07K13/00; C12N5/10; C12P21/08
G01N33/68; A61K39/395; C12Q1/68

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K ; A61K ; G01N C12Q

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	SCIENCE vol. 257, 28 August 1992, LANCASTER,PA pages 1248 - 1251 Mountjoy KG;Robbins LS;Mortrud MT;Cone RD; 'The cloning of a family of genes that encode the melanocortin receptors.' see the whole document	1-6,9-14
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 88, no. 19, 1 October 1991, WASHINGTON US pages 8525 - 8529 Mertz LM;Catt KJ; 'Adrenocorticotropin receptors: functional expression from rat adrenal mRNA in Xenopus laevis oocytes.' see the whole document	1,6

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
26 AUGUST 1993	23 -09- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NAUCHE S.A.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 82, March 1985, WASHINGTON US pages 1372 - 1375 BOST, K. L. ETAL.; 'Similarity between the corticotropin (ACTH) receptor and a peptide encoded by an RNA that is complementary to ACTH mRNA' see the whole document	1,6,15, 31-45
X	MOLECULAR AND CELLULAR ENDOCRINOLOGY vol. 44, 1986, pages 1 - 9 BOST, K.L. ET AL.; 'Molecular characterization of a corticotropin receptor' see the whole document	1,6,15
A	BIOCHEMISTRY. vol. 25, no. 6, 25 March 1986, EASTON, PA US pages 1339 - 1346 HOFMANN K;ROMOVACEK H;STEHLE CJ;FINN FM;BOTHNER-BY AA;MISHRA PK; 'Radioactive probes for adrenocorticotropic hormone receptors.' see the whole document	21-30
X	PEPTIDE RESEARCH vol. 2, no. 3, 1989, pages 213 - 220 EBERLE, A.N. ET AL.; 'Receptor-specific antibodies by immunization with 'antisense' peptides?' See the introduction	1,31